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Further Studies on Glucophylline

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FURTHER STUDIES ON GLUCOPHYLLINE*

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IN A PREVIOUS report on the diuretic activity of glucophylline by Maloney, Burton, and Robinson,[1] in which experimental data were presented regarding the action of this double salt of theophylline and methylglucamine, a xanthine compound exhibiting the very desirable properties of high diuretic efficiency with long duration of action, it was indicated that our study was being continued with the object of determining "whether glucophylline exerts any untoward effects on the liver or kidney." The present report sets forth these and other pertinent results obtained from this investigation. The study was conducted in three parts, viz., the effect on (1) weight, (2) blood, and (3) vital organs. Full-grown dogs and immature rabbits were employed.

1. Weight. A. Dogs: Nine full-grown dogs, ranging in weight from 8.2 to 11.5 kg., were divided into groups of three and treated with physiologic saline (controls), methylglucamine, and glucophylline, respectively. All the animals lost weight during the course of the fifty-four days of the experimental period. The percentage loss in each group was 6.7, 9.3, and 11.6 in the order named. We do not think the medicaments played any significant role. The determinants here were probably the new diet and the unaccustomed artificial environment of the kennels (see Table I).

B. Rabbits: Thirty-six female rabbits, ranging in weight from 0.8 to 1.7 kg., were employed. These were divided into three groups of twelve. Half of the animals in group 1 received no treatment whatever, while the other half received normal physiologic saline. These were used as two sets of controls. Those in groups 2 and 3 received methylglucamine and glucophylline, respectively. For convenience, all animals received equal volumes of the substances injected: the rabbits received 0.2 c.c. for the first period of thirty days and 0.4 c.c. for the second period of thirty days; the dogs, 1 c.c. throughout the duration of the treatment. The methylglucamine was prepared as a 6.2 per cent solution, so that each rabbit received 12.4 mg. and each dog 62 mg. of this salt per dose; the glucophylline was prepared as a 10 per cent solution (in the formula the ratio of methylglucamine to theophylline is 1:16 to 1:18), so that each rabbit received approximately 10 mg. for thirty days and 20 mg. later, and each dog received approximately 50 mg. of each of the two constituents in the compound. On the basis of the initial weight the milligram per kilogram dose was then computed for each animal. This figure appears in the respective tables. Injections were all made intraperitoneally once daily. All the animals in the two species were kept under similar environmental conditions

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TABLE I

WEIGHT CHANGES IN DOGS

GROUP	ANIMAL	DOSE TOTAL	INITIAL WEIGHT (KG.)	54 DAYS FINAL WEIGHT (KG.)	% DECREASE	WEIGHT LOSS (KG.)
Control (Saline)	1	1 c.c.	8.3	7.7	7	0.6
	2	1 c.c.	11.4	10.9	4	0.5
	3	1 c.c.	11.1	10.1	9	1.0
Methylglucamine hydrochloride	4	6 mg./kg.	10.3	10.2	1	0.1
	5	6.7 mg./kg.	9.2	8.0	13	1.2
	6	5.4 mg./kg.	11.5	9.9	14	1.6
Glucophylline	7	10.8 mg./kg.	9.2	6.93	25	2.3
	8	10.1 mg./kg.	9.9	9.6	3	0.3
	9	10.3 mg./kg.	9.8	9.1	7	0.7

and were provided at all times with an excess of food and water. Weights were recorded at weekly intervals. With the exception of one control rabbit, which died on the twenty-fourth day of the experimental period, all animals lived to the end and without exception appeared to maintain normal health throughout. The dogs in the glucophylline group (No. 3) were definitely more active and lively than the others. It is of interest to note that the animal caretaker, who had no knowledge of what the animals being injected were receiving, volunteered the observation that the group of rabbits which we were treating with glucophylline ate more food and drank more water than those making up the other group; and that the amount of feces and urine voided by these animals was considerably greater. Reference to Table II reveals a fairly uniform average percentage of weight gains for the several groups both from the initial doses and after the doses were doubled. In the first series the order of percentage increases for the thirty-day period was: no medication, 47; physiologic saline, 59; methylglucamine, 49.5; glucophylline, 49. By the end of the first thirty days, then, the animals in all groups had made substantial weight gains, some of them better than 50 per cent. This, no doubt, accounts for the gradual tapering off of weight increases during the second phase of the experiment when the doses were doubled. Most of the animals had already reached maturity, after which weight increase was due primarily to deposition of fat and only secondarily to actual body growth. The total group average percentage gains were: no medication, 136; saline, 150; methylglucamine, 139; and glucophylline, 128.

2. Blood: Studies on blood changes included (1) erythrocytes, (2) white blood cells, (3) differential, (4) nonprotein nitrogen, and (5) creatinine. One complete weekly determination was made on each dog during the fifty-four days of the experimental period. In the case of the rabbits there was one complete weekly determination on each of eight animals, two within each group, the last being made at the time of disposition of the animal for histologic purposes. As with the dogs, the figures appearing in Table III represent the composite averages within each group. Included also in this table, for purposes of comparison, are figures of normal blood values for dogs and rabbits.*

*Taken from figures compiled by Wagoner and Custer and reproduced in Tables I and III, pages 33 and 34 of their *Handbook of Experimental Pathology*, Springfield, Ill., 1932, Charles C Thomas.

TABLE II

WEIGHT CHANGES IN RABBITS

GROUP	ANIMAL	DOSE TOTAL	INITIAL WEIGHT (KG.)	WEIGHT IN 30 DAYS (KG.)	% INCREASE	DOSE TOTAL	WEIGHT ON FINAL DAY (KG.)	TOTAL INCREASE WEIGHT (KG.)	TOTAL % INCREASE
Control (a) No medication	1	0	0.88	Died					
	2	0	0.93	1.81	95	0	2.86	1.94	179
	3	0	0.98	1.74	78	0	2.61	1.64	168
	4	0	0.98	1.60	65	0	2.02	1.04	107
	5	0	1.05	1.66	57	0	2.59	1.54	146
	6	0	1.23	1.08	−12	0	2.20	0.97	80
(b) Physiologic saline	1	0.2 c.c.	1.23	1.82	49	0.4 c.c.	2.60	1.38	112
	2	0.2 c.c.	1.18	1.84	55	0.4 c.c.	2.55	1.38	116
	3	0.2 c.c.	1.15	1.76	53	0.4 c.c.	2.57	1.42	123
	4	0.2 c.c.	1.05	1.75	66	0.4 c.c.	2.60	1.55	147
	5	0.2 c.c.	0.95	1.58	66	0.4 c.c.	2.82	1.87	197
	6	0.2 c.c.	0.80	1.32	65	0.4 c.c.	2.48	1.68	209
Methylglucamine hydrochloride		mg./kg.				mg./kg.			
	1	8.3	1.50	2.08	39	11.6	3.22	1.72	115
	2	8.4	1.48	2.27	54	10.8	2.50	1.02	70
	3	9.0	1.38	2.02	47	12.3	2.60	1.22	89
	4	10.6	1.18	1.35	15	17.7	2.70	1.53	130
	5	10.8	1.15	1.79	55	13.9	2.74	1.59	140
	6	11.0	1.13	1.16	2	21.6	2.36	1.24	110
	7	11.5	1.08	1.33	23	19.1	2.33	1.26	110
	8	11.8	1.05	1.60	52	15.5	2.43	1.38	130
	9	12.1	1.03	1.50	46	16.5	1.92	0.89	81
	10	12.7	0.98	1.56	60	15.5	3.16	2.19	225
	11	13.1	0.95	1.54	62	16.4	2.57	1.62	170
	12	15.5	0.80	1.82	137	13.8	3.26	2.46	300
Glucophylline	1	11.5	1.75	2.24	21	17.9	3.56	1.81	103
	2	12.5	1.60	2.34	45	17.1	3.25	1.65	103
	3	14.3	1.40	2.05	45	19.5	2.82	1.62	100
	4	14.3	1.40	2.12	50	18.9	3.24	1.84	132
	5	16.1	1.24	1.95	57	20.5	2.95	1.71	137
	6	16.7	1.20	1.96	63	20.4	2.67	1.47	121
	7	16.7	1.20	1.82	50	21.9	2.76	1.56	130
	8	16.7	1.20	1.62	35	24.9	2.65	1.45	120
	9	17.4	1.15	1.62	42	24.9	2.49	1.34	116
	10	18.2	1.10	1.58	44	25.0	2.79	1.69	153
	11	18.2	1.10	1.78	62	22.2	2.93	1.83	166
	12	20.0	1.00	1.73	73	23.1	2.52	1.52	150

TABLE III

COMPOSITE BLOOD VALUES OF DOGS AND RABBITS

ANIMAL VALUES	R.B.C. MILLIONS	W.B.C. THOUSANDS	DIFFERENTIAL PERCENTAGE					HB. %	N.P.N. %	CREATININE %	NUMBER OF SAMPLINGS
			NEUT.	LYMPH.	MONO.	EOS.	BASOPH.				
Dog: "Normal values"*	7.2	11.8	69	20	6	5	0.7	77	30.8	1.5	?
Saline	5.5	6.7	71	25	4	4	0	111	32.4	1.4	21
M-glucamine	5.3	8.5	70	26	6	0	0	109	26.1	1.3	21
Glucophylline	5.4	10.0	77	21	2	0	0	103	28.9	1.5	20
Rabbit: "Normal values"*	5.6	7.9	43	42	9	2	4.0	95	31.0	1.1	
No medication	6.3	7.5	17	66	3	13	1	100	30.7	1.6	11
Saline	5.9	6.7	15	60	4	21	0	102	46.9	2.0	12
M-glucamine	5.5	8.1	10	68.5	5	16	0.5	104	38.1	2.0	20
Glucophylline	5.0	7.4	22	63.0	4	11	0	99	39.5	2.0	20

*"Normal values" of blood (count and chemistry) reported above are taken from Tables I and III, respectively, from Wagoner, G., and Custer, R. P.: *A Handbook of Experimental Pathology,* Springfield, Ill., 1932, Charles C Thomas.

TABLE IV

TISSUE CHANGES IN DOGS AND RABBITS

SPEC. ANIMAL	BRAIN	LUNG	HEART	LIVER	KIDNEY	SPLEEN
A. Physiologic Salt Solution						
10. Dog 2	No change	No change	Slight cloudy swelling	Slight cloudy swelling	Slight cloudy swelling	Slight pigmentation. Slight increased cellularity in pulp
15. Rabbit A-4	No change	Hyperemia, small focal hemorrhages	Focal areas of necrosis with considerable inflammatory reaction	Cloudy swelling	Degeneration of tubular epithelium, especially in convoluted tubules	Slight hyperplasia
17. Rabbit A-3	No change	Slight hemorrhages	Cloudy swelling	Slight cloudy swelling	Hyperemia; cloudy swelling	Marked hyperemia
B. Methylglucamine						
7. Dog 4	No change	No change	Cloudy swelling	Marked cloudy swelling	Cloudy swelling	Hyperplasia
8. Dog 5	No change	Diffuse hemorrhages throughout	No changes	Cloudy swelling	Cloudy swelling, marked in some tubules; necrosis in some tubules	Hyperplasia
11. Rabbit B-1	--	Diffuse hemorrhages into alveoli, bronchi, and interstitial tissue	Marked hyperemia; cloudy swelling	Cloudy swelling	Hyperemia; cloudy swelling	
19. Rabbit B-8	No change	Small hemorrhages	Cloudy swelling	Cloudy swelling; increase in periportal connective tissue	Marked hyperemia; cloudy swelling	Hyperemia
13. Rabbit B-12	Slight thickening of meninges. Focal hemorrhage—focal area of softening with cellular reaction	Small hemorrhages; arteriosclerosis	Multiple hemorrhages; cloudy swelling	Cloudy swelling	Cloudy swelling. Leucocytic infiltration in interstitial tissue of cortex. Hyperemia	Hyperemia
5. Rabbit B-3	No change	Hyperemia; arteriosclerosis	Small hemorrhages. Slight increase in perivascular connective tissue	Cloudy swelling; passive hyperemia; maybe some fat	Cloudy swelling	Slight hyperplasia

TABLE IV—CONT'D

SPEC. ANIMAL	BRAIN	LUNG	HEART	LIVER	KIDNEY	SPLEEN
			C. Glucophylline			
9. Dog 9	No change	Hemorrhages in focal areas, cells with vacuolated cytoplasm in interstitial tissue. Suggest fat, slight fibrosis	Cloudy swelling	Cloudy swelling	Cloudy swelling	No change
12. Rabbit C-7	Hyperemia; infiltration of small and large monocytes in meninges	Small granuloma	Slight cloudy swelling	Cloudy swelling	Cloudy swelling; hyperemia; arteriosclerosis of small arteries	Slight hyperplasia
14. Rabbit C-2	No change	Diffuse hemorrhages	Cloudy swelling	Cloudy swelling	No change	Hyperplasia
18. Rabbit C-5	No change	Hyperemia, few small hemorrhages	Cloudy swelling; small hemorrhages	Cloudy swelling	Marked cloudy swelling; hyaline casts in collecting tubules	Slight hyperplasia

3. Histologic: Tissues fixed in 10 per cent formalin were prepared for microscopic study from both sets of animals. The dogs were all disposed of on their last experimental day (the fifty-fourth); the rabbits were disposed in series of twos at ten-day intervals, beginning with the end of the first experimental period on the thirtieth day. Thus, whereas the first group that were treated had received a single daily dose for thirty days, the second, third and fourth groups received this, followed afterward by a double dose daily at intervals of ten, twenty, and thirty days, respectively. Although these animals were exposed to unequal quantities of the drugs over varying periods of time, no appreciable differences could be demonstrated in the tissues studied. Pulmonary hemorrhages, demonstrable in most samples studied, were due to ante-mortem traumatic injury. Samples of brain, lung, heart, liver, kidney, and spleen were examined (see Table IV).

From these studies it is apparent that glucophylline neither disturbs the growth nor checks the normal increase in the weight of immature rabbits. Furthermore, with respect to both dogs and rabbits no noticeable difference could be observed in the general appearance of treated and untreated animals regarding health and general state of nutrition. In these respects all the groups exhibited the same general condition of health. The microscopic study revealed the presence of cloudy swelling as a rather constant finding in samples taken from both dogs and rabbits. This finding was quite as conspicuous in the tissues of animals used as controls, as it was in those of animals treated with methylglucamine and glucophylline. The blood samples taken from our rabbits showed an inordinately high differential eosinophilic count. This phenomenon

is a matter of not uncommon occurrence in the blood of laboratory animals. It is very probable that these cells, or many of them, were pseudo-eosinophiles or emphophiles. Referring to this type of cell, Wagoner and Custer[2] make the observation that "the analogue of the human neutrophile varies only in that the granules are larger than neutrophilic granules and take the acid stain by preference, although there is a slight affinity for the basic dye." Since this eosinophilic condition was uniformly present in each group, treated as well as untreated, we felt that we were dealing with cases of pseudo-eosinophilia and that these findings, therefore, were probably of no pathologic significance. Indeed, many a laboratory animal that appears to be normal and healthy in every respect has been found to exhibit visceral lesions exposed by the microscope, if not grossly visible at autopsy. In the course of these studies one of the female dogs in our kennels gave birth to a litter of six pups. On the day after birth we began giving daily injections of glucophylline to three of these pups, using the other three as controls. No significant differences have been noticed, and at the end of thirty days the relative individual weights have shown uniform increases.

SUMMARY

Laboratory studies on dogs and rabbits treated with methylglucamine and glucophylline revealed:

1. Growth of immature rabbits is not disturbed. These animals made increases in size and weight commensurate with normal controls.
2. The dogs, treated and controls, all lost weight—a condition due probably to food and housing restraint.
3. Histologic study of brain, heart, lung, spleen, kidney, and liver of both dogs and rabbits showed no harmful effects that might be attributed to the drug.
4. Significant figures setting forth results are appropriately tabulated.

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REFERENCES

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